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A BIMODAL RESPONSE TO DIETARY SUGARS BY AN INSECT[1]

STANLEY D. BECK

Department of Entomology, University of Wisconsin, Madison 6, Wisconsin

The gustatory responses of different animal species to sugars have been studied by many workers, and the apparent universality of animal acceptance of sucrose has been pointed out by Frings (1946). The biological importance of carbohydrates and their lack of olfactory-stimulating properties makes them well suited for experimental studies of gustatory responses. As discussed by Roeder (1952), insects are excellent experimental animals for studies in several areas of sensory physiology, owing to their small size, ease of culturing in large numbers, and relative simplicity of behavior. Several different aspects of the gustatory reactions of insects to sugars have been investigated: taste-acceptance thresholds have been determined; the influence of sugars on feeding behavior has been studied; and the relationships between nutritive value and taste-acceptability have been investigated (for review see Dethier, 1953).

As part of a study of the behavior of the larva of the European corn borer, *Pyrausta nubilalis* (Hübn.), on its principal host plant, an investigation was undertaken of the influence of dietary sugars on larval feeding behavior under controlled experimental conditions. The larvae were found to be very sensitive to sugars (Beck, 1956a), and the concentration of sugars in the tissues of the corn plant was shown to influence the orientation of the insect on the plant (Beck, 1956b). The experimental method developed during the course of this research made possible the quantitative measurement of the influence of gustatory substances on feeding behavior. The nature of the measured feeding response and a discovered bimodality of the insect's response to low concentrations of sugar constitute the subject matter of the present report.

Methods

The experimental method employed was of a multiple choice design, in which newly hatched, unfed European corn borer larvae were allowed to choose the dietary media on which to feed. The agar-based medium shown in Table I was used as the nutritional substrate. Agar-gel alone was of a consistency unacceptable to the insects; the addition of casein and cellulose resulted in a medium on which

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the larvae would feed. The amounts of sugars employed in these experiments were far too minute to necessitate the adjustment of the amounts of the other constituents to compensate for the slight changes in volume and concentration.

The media were mixed thoroughly with a motor-driven stirrer while being held in a boiling water bath, and then poured into Petri plates (15 mm. × 90 mm.) to gel. From the gelled media, round discs were cut by means of glass tube 18 mm. in diameter. The discs were transferred to Petri plates where they were placed at equal distances apart in a circle, the center of which was the center of the dish. The discs of diet so placed are referred to hereafter as feeding stations. In two-choice experiments there were four feeding stations per Petri dish, each medium being represented twice. The similar media were placed opposite each other in the four-membered circle. In four-choice experiments, eight feeding stations were present, each medium being represented at two stations, and the relative positions of the different media were randomized according to a table of random numbers. The position-randomization of the media in four-choice experiments was modified only to the extent that two identical diets were never placed adjacent to each other. This modification of the random distribution was found to be necessary in order to avoid position effect errors. About 40 European corn borer eggs in the black-head stage (and about four hours from hatching) were placed on a small piece of moist blotting paper at the center of dish. The lids of the Petri dishes were lined with tightly fitting discs of blotting paper to aid in preventing the larvae from escaping. Eight replicate Petri dishes were set up in each experiment. After the experiment was set up, the dishes were placed in the dark at 30° C. for 24 hours. At the end of this time, the dishes were examined and the number of larvae established at each feeding station was recorded. No counts were made of larvae not on feeding stations. The counts of the number of larvae established at each feeding station were interpreted as reflecting the influence of the experimental diets on the maintenance of larval feeding over the experimental period. Preliminary experimentation demonstrated that the distribution of larvae among the feeding stations was random when the feeding stations were of identical diets.

Table I

Composition of agar-based media used to test the feeding reactions of newly-hatched larvae of the European corn borer

Substance	Amount (g)
Bacto-agar	1.10
Fibrous cellulose	0.50
Powdered cellulose	2.00
Casein, vitamin free	3.00
Glucose (or other sugar)	variable
Distilled water	40.00 (ml.)

The physical characteristics of the various media were found to be quite uniform. The larvae were found to be relatively insensitive to small differences in pH and water content; small variations in these properties did not represent a source of experimental error. It was found unnecessary to maintain aseptic conditions in the Petri dishes when the incubation period was 24 hours. With a 48-hour incubation period, the use of aseptic techniques was necessary. However, the larval establishment data at 24 hours were indistinguishable from those at 48 hours, so the shorter time and simpler technique were adopted.

The experimental data were treated statistically by analysis of variance, after being subjected to square root transformation as recommended by Snedecor (1946) for treatment of small numbers. Regressions were calculated by the method of least squares.

Results and Discussion

The nature of response to sugar. Four-choice experiments, in which the larvae were offered dietary media containing different amounts of glucose, yielded re-

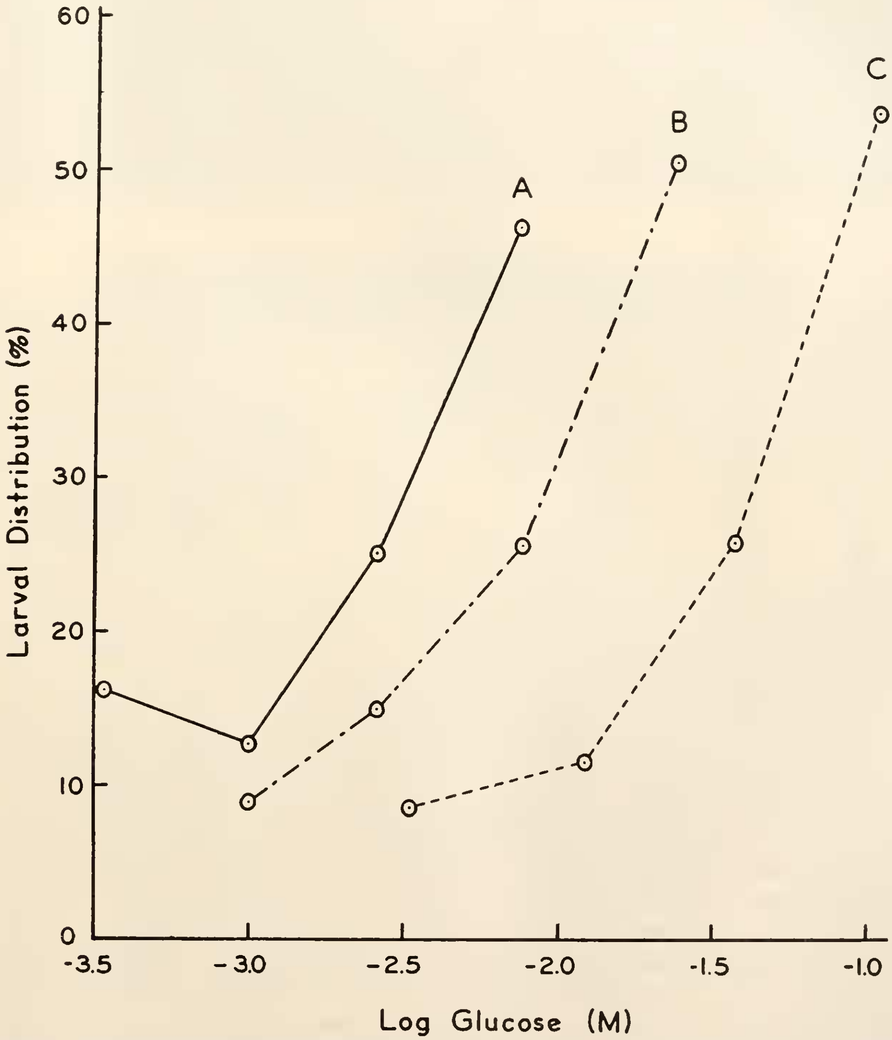

Figure 1. Three experiments (A, B, and C) on the effect of glucose concentration on the distribution of European corn borer larvae among dietary media (four-choice experiments).

sponse curves such as those shown in Figure 1. Over the range of glucose concentrations tested, the larvae tended to concentrate on the highest sugar level available to them. Similar response curves were obtained with sucrose and fructose. The percentage of the larvae established on a given feeding station was apparently independent of the amount of glucose actually present in the medium, but was dependent upon the concentration relative to the concentrations offered in the other media present.

Early in the investigation it became obvious that interpretation of response data would be dependent upon an understanding of the mechanisms by which the larval population became distributed among the feeding stations in proportion to the relative sugar content of the media. Observations on the behavior of newly hatched borer larvae showed that they were initially attracted to the agar-based media, even when the media were devoid of sugars or other nutrients. Such behavior was probably the result of a hydrotactic response. It was also observed that the larvae were quite active up to at least 24 hours after hatching and tended to wander from feeding station to feeding station. They displayed much greater tendency to wander away from stations low in sugar than from those containing

TABLE II

The effect of time of observation on determinations of the influence of glucose on the establishment of newly hatched European corn borer larvae

Time after eclosion (hr.)	Larval establishment		Statistical significance of difference
	control—no glucose (%)	glucose 0.004 *M* (%)	
2	49.3	50.7	not significant
6	29.1	70.9	highly significant
12	28.6	71.4	highly significant

high sugar levels, however. Because of the larvae's sensitivity to light, it was not possible to determine by direct observation the exact influence of sugar concentration on the duration of larval feeding.

On the basis of the observations on larval behavior and the relative nature of the experimental data, the hypothesis that the length of time that a larva remains at a given feeding station is in proportion to the sugar concentration in that station was tested experimentally. Experiments were run in which the effect of the presence or absence of glucose on the distribution of the larvae between two media was measured at different times after the eggs hatched. The results of such two-choice experiments are shown in Table II. The data show that the initial larval distribution between the media was random. By six hours after hatching, the random distribution had been replaced by a non-random distribution in which most of the larvae were found on the feeding stations containing glucose. Although the percentage distribution remained materially constant after six hours, the larvae tended to wander intermittently up to well over 24 hours. At about six hours after hatching, the larval distribution had reached an equilibrium, in which the number of larvae vacating a given feeding station per unit time was equal to the number arriving from other stations. If the ratios of larval distribution among the media are directly related to the ratios of the average times the larvae remained on the different media, it should be possible to reproduce the experimental data by calculating the equilibrium in time. Table III shows such a calculated distribution equilibrium. Very good agreement between observed and calculated data was obtained. It is apparent that response data, such as shown in Figure 1, represent larval distributions at equilibrium. The percentage distribution of the larvae at the point of equilibrium must be relative to the single experimental variable of sugar concentration, and the distribution data are in agreement with the hypothesis that the response data measure the ratios of the average larval feeding times on the different sugar concentrations.

The general relationship between dietary sugar (glucose) concentration and duration of larval feeding was estimated by the assumption of arbitrary time units in which the average larval feeding time on a just subliminal concentration (0.001 M) of glucose was taken as one feeding time unit. Feeding time values were then calculated from larval distribution ratios obtained in a series of two- and four-choice experiments. The regression of feeding time on glucose concen-

TABLE III

Comparison of larval distribution observed in a four-choice experiment with calculated distribution equilibrium

Feeding stations:	A	B	C	D
Larval distribution *observed* (%):	13.0	17.5	27.5	42.0
Distribution ratio (DR) *observed*:	1.00	1.38	2.15	3.38
Larvae leaving station per unit time (1/DR×100) *calculated* (%):	100	72.5	46.5	29.6
Larval distribution at equilibrium *calculated* (%):	12.7	17.5	27.6	42.2
Larval distribution difference (*observed—calculated*):	0.3	0.0	−0 1	−0.2

tration obtained by this method is shown in Figure 2. The value of the feeding time unit, as here defined has not been accurately determined, and the best approximation now possible is that it lies between 20 and 45 minutes. Its value in conventional time units is of little importance, as it will, undoubtedly, change with varying experimental conditions.

The importance of feeding time in the response of insects to sugars is indicated by the published results of several other workers. Kunze (1927) found that the duration of feeding by honey bees could be used as an index of the acceptability of sugar solutions to this insect. Weis (1930) reported that the length of time of uninterrupted feeding was proportional to the sugar (sucrose) concentration of the liquid fed upon by the butterfly *Pyrameis atalanta* L. The volume of liquid ingested per feeding by the honey bee was observed to be directly proportional to the dietary concentration of sucrose (Von Frisch, 1935). Dethier and Rhoades (1954) described certain relationships between the volumes of liquid ingested by adult blowflies and the sugar content of the liquids employed. Using adult blowflies, Frings and Frings (1946) demonstrated that the volume ingested is directly proportional to the duration of feeding. The tendency for an organism to concentrate its feeding on nutritional substrates high in sugar content has been termed *saccharotrophism* (Beck, 1956b).

From the results presented above, it is apparent that saccharotrophism in the European corn borer, and probably in some other insects, is the influence of sugar concentration on duration of feeding. The experimental data constitute quantita-

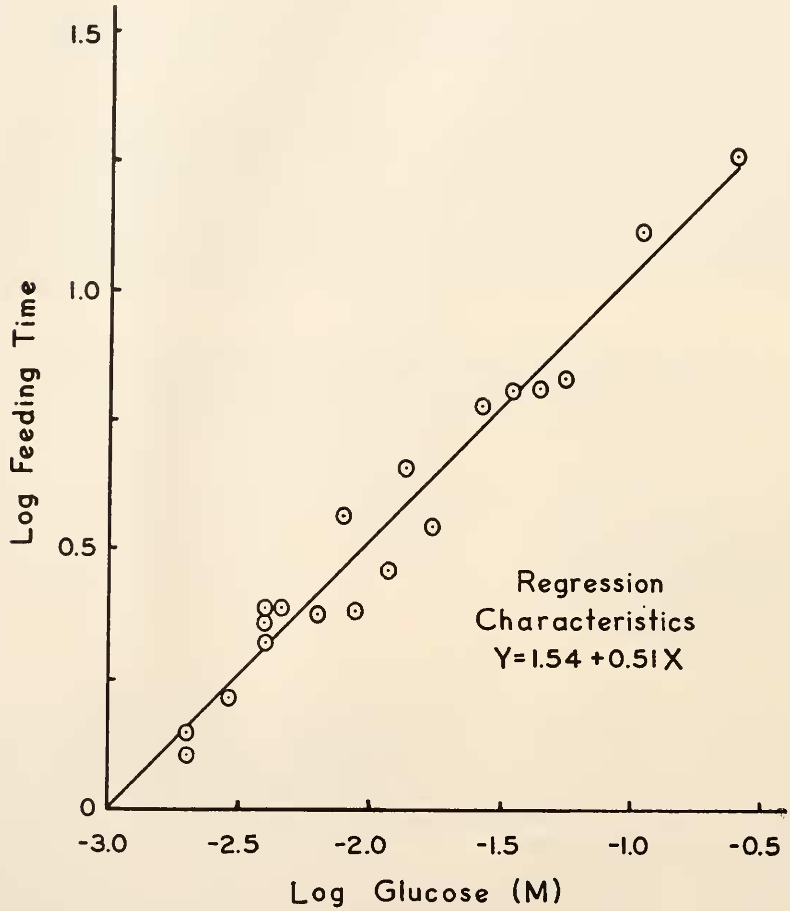

FIGURE 2. The effect of glucose concentration on the duration of feeding by newly hatched larvae of the European corn borer.

tive measurements of the intensity of the saccharotrophic response. The data are not a measure of the proportion of the insect population responding to the sugar concentrations tested. Weis (1930) questioned the reliability of using feeding duration as a quantitative measure of acceptability because of the uncontrolled variables of age, water balance, and nutritional history of the experimental animals. In the present study these factors were standardized by the use of newly-hatched and previously unfed larvae, and under these conditions the European corn borer larvae employed represented a nearly ideally uniform experimental population.

Bimodal response. During the course of running a series of four-choice feeding experiments designed to allow an estimation of the feeding acceptance thresholds for glucose, sucrose, and fructose, response curves similar to curve A of Figure 1 were frequently obtained. Such curves, in which the response to the lowest sugar concentration was higher than to the next higher concentration, were at first considered to be the result of the statistical vagaries of the experimental method. As more data were obtained, it became evident that responses of this nature were consistently observed in any experiment which included two sugar concentrations somewhat below the apparent acceptance threshold. This phenomenon was investigated in greater detail by means of a series of two-choice experiments in which one of the pair of diets used was devoid of sugars and the other contained a known concentration of glucose. The results are shown in Figure 3. Each point shown in this figure is the mean of the results of from three to five experiments, each of which was made up of eight replicates. Statistical analyses of the data showed that the response to the diets containing sugar was significantly different from the response to the control diets at all sugar concentrations except 0.0001 M (log = − 4.0) and 0.001 M (log = − 3.0). At concentrations below 0.001 M but above 0.0001 M, the larvae tended to concentrate their feeding on the control diets (no sugar) in preference to the diets containing such low levels of sugar. Although the response differences were small, they were reproducible and statistically significant. These data indicate a bimodal response to glucose concentration. At concentrations above an apparent threshold of approximately 0.001 M, the feeding response (time) was directly proportional to the sugar concentration and the data are consistent with the Weber-Fechner law. The larval reaction toward concentrations lower than 0.001 M was opposite in sign, in that it reduced the feeding period below that elicited by diets containing no sugar. The threshold for the negative response appeared to lie between 0.0001 and 0.0002 M. The negative response toward concentrations below the threshold for a positive feeding response was observed in experiments with fructose and sucrose, as well as with glucose. It is therefore apparent that it is not a reaction associated with glucose only. Different manufacturer's lot numbers of glucose (A.C.S. specifications) gave identical results, so the reaction was not a response to the purity of the sugar preparations.

The physiological mechanisms underlying the observed bimodality have not been determined. The data do not necessarily imply a bimodality of response of sugar-sensitive receptor organs, as the present investigation has dealt with only behavioral aspects of the problem. The data show that: (1) the response of European corn borer larvae to different concentrations of sugars is measurable and can be expressed quantitatively in terms of the effect of sugar concentration on the duration of larval feeding. (2) As the sugar concentration is increased above a threshold value lying between 0.0001 M and 0.0002 M, there is a proportionate increase in the duration of feeding. (3) The response data show a bimodal characteristic in that larval feeding on diets containing low suprathreshold concentrations of sugar is of shorter duration than on diets containing subthreshold amounts of sugar. The following interpretation of these findings is offered, although its speculative nature must be recognized. The duration of feeding is probably related to the time required for the insect to become adapted to the diet. When in contact with a diet containing no sugar (or a subliminal concentration), stimulation of sugar receptors does not occur, and these sense organs can play no role in determining feeding duration. When they are stimulated, however (as by a suprathreshold sugar concentration), feeding maintenance is dominated by the impulses they feed into the nervous system. Such impulses cease as the larva becomes

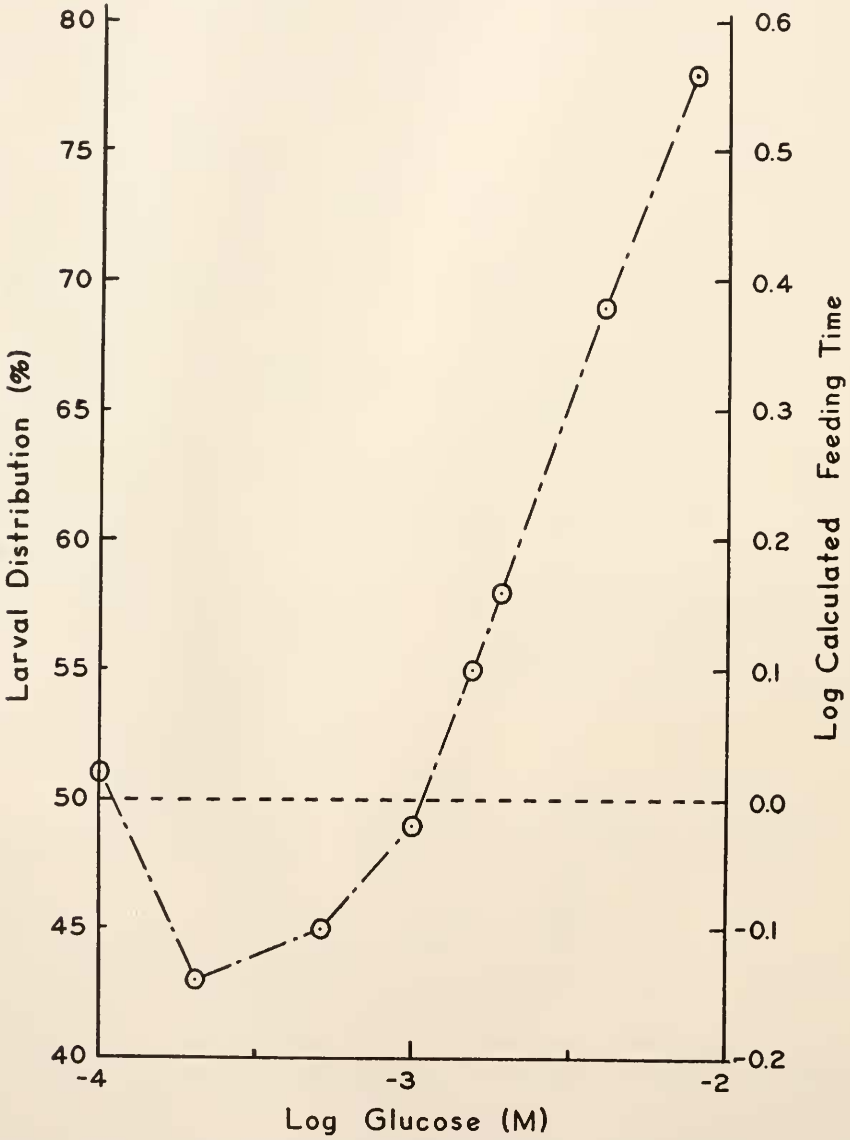

FIGURE 3. Feeding response spectrum of European corn borer larvae for glucose. Two-choice experiments. Plotted data do not include responses to control (no sugar) media.

adapted to the diet, and the behavior pattern undergoes a shift. The lower the sugar level, the more rapid the adaptation, and the shorter the feeding period. In the absence of supra-threshold sugar concentrations, duration of feeding must be determined by other factors. A bimodal response would be obtained experimentally under conditions where duration of feeding is more sensitive to sugar than to alternative factors influencing behavior in the absence of sugars.

A bimodal response to low sucrose concentrations has been reported in human tests by Neuman (1944). Working with human subjects, she found that the quality of sensation caused by sucrose was dependent upon intensity of the stimulus. Her subjects reported that the perceived sensations from sucrose solutions near the threshold level of concentration may be a complex of sweet, bitter, and/or sour. Samuel Renshaw (personal communication) determined human taste spectra for sucrose and found that at the lowest threshold, sucrose gives rise to a smooth tactual sensation rather than to a sweet taste. As the concentration is progressively increased, the taste is bitter, then bitter-sweet, and finally purely sweet. Although it is not possible to determine the quality of sensation produced in an experimental animal, the response data obtained by use of European corn borer larvae show a response spectrum to sugars that is strikingly parallel to the human taste spectrum for sucrose.

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Summary

1. A study of the feeding behavior of newly hatched, previously unfed larvae of the European corn borer has shown that dietary sugar concentration has a pronounced and quantitatively measurable effect on such behavior.

2. The larvae tended to prolong their feeding in proportion to the dietary sugar concentrations.

3. The responses to sugar concentrations above a positive response threshold of about 0.001 M were in agreement with Fechner's law over the concentration range tested. There was a small but significant negative feeding response toward sugar concentrations below 0.001 M and down to 0.0002 M.

4. The experimental results obtained indicate that this organism displays a bimodal response to glucose, sucrose, and fructose, in which the modes differ in sign and are dependent upon the concentrations employed. The response spectrum of the insect toward the sugars tested appears to be parallel, if not directly comparable, to the human taste spectrum for sucrose.

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